

Applicant : David Alland et al.
Serial No. : 09/918,951
Filed : July 31, 2001
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computer readable form to be used herein is identical to the enclosed Sequence Listing, and does not introduce new matter into the application as filed.

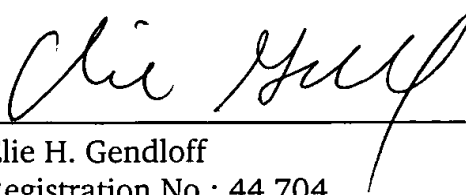
Applicants have also amended the specification to include the correct sequence identifiers for sequences recited therein, as required by the Sequence Rules.

In light of the above, applicants respectfully submit that they have complied with the Sequence Requirements and all other requirements to comply with the Notice to File Missing Parts of Nonprovisional Application of September 25, 2001 in this case. Applicants therefore respectfully request examination of the claims as amended. Should there be any additional matters that prevent examination of the claims, the Examiner is urged to contact the undersigned attorney.

Respectfully submitted,

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December 20, 2001

By: 
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Appendix A

Marked Up Amended Specification Paragraphs and Claims - Application 09/918,951
Additions are underlined and deletions are bracketed

IN THE SPECIFICATION

Paragraph at page 5, line 22 - 27

Figures 6A-6C: Figures 6A-6C set forth the nucleic acid sequences of the iniB, iniA and iniC genes, and the promoter region of the iniB gene. MTCY279, genebank accession Z97991. Nucleotides 9048-9101 (SEQ ID NO:1), then nucleotides 1 - 159 of *M. tuberculosis* cosmid MTY13E10 (nucleotides 1-159 of SEQ ID NO:2), genebank accession Z95324. For a total of 213 nucleotides. Nucleotide sequences of genes, numbering from MTY13E10 iniB 160-1559 (nucleotides 160-1559 of SEQ ID NO:2); iniA 1636-3558 (nucleotides 1636-3558 of SEQ ID NO:2) and iniC 3555-5036 (nucleotides 3555-5036 of SEQ ID NO:2).

Paragraph at page 6, lines 1 - 2

Figure 7: Figure 7 sets forth the amino acid sequences encoded by the iniB (SEQ ID NO:3), iniA (SEQ ID NO:4), and iniC (SEQ ID NO:5) genes.

Paragraph at page 12, lines 8 - 15

The vector constructs of the present invention contain a nucleotide sequence encoding suitable regulatory elements so as to effect expression of the vector construct in a suitable host cell. Those skilled in the art will appreciate that a variety of [enchancers] enhancers, promoters, and genes are suitable for use in the constructs of the invention, and that the constructs will contain the necessary start, termination, ribosomal binding sequences, and control sequences for proper transcription and processing of the iniB promoter region when the vector construct is introduced into a host cell.

Paragraph at page 15, line 13 - page 16, line 10

Creation of ribosomal free customized amplification libraries. One

thousand cosmid library clones were inoculated into "master" 96 well microtiter plates containing L broth and ampicillin 50 μ g/ml, transferred by a pronged "frog" onto Biotrans nylon membranes (ICN Pharmaceuticals, Costa Mesa, CA), and hybridized separately with [α^{32} P] radiolabeled (Megaprime labeling kit, Amersham, Arlington Heights, IL) PCR probes to *M. tuberculosis* ribosomal 5S, 16S, and 23S genes. Fourteen cosmids containing ribosomal DNA were identified; non-ribosomal cosmids were re-inoculated from master plates and individually cultured. Cosmids were extracted by SDS/alkaline lysis (17) in pools of 16. Cosmid DNA was pooled, digested with *PacI*, which does not restrict the *M. tuberculosis* genome, and insert DNA was purified from an agarose gel by electro-elution.

Approximately 1 μ g of precipitated DNA was digested with *AluI* and 100 ng run on a 2% NuSieve GTG low melting point agarose gel (FMC Bioproducts, Rockland, ME). Marker DNA was run simultaneously in a separate gel to avoid cross contamination of samples. The gels were aligned, and the section corresponding to 400 - 1,500 base pairs of the *AluI* digest was excised. Five μ l of gel slice was ligated with 1 μ l of Uniamp *XhoI* adapters 2 pmol/ μ l (Clontech, Palo Alto, CA) in 20 μ l total volume. Ten μ l of the ligation was PCR amplified with 2 μ l of 10 μ M Uniamp primers (Clontech), 1X vent polymerase buffer and 0.8 units of Vent (exo-) polymerase (New England Biolabs, Beverly, MA) in 100 μ l total volume. After a five minute hot start, ten cycles of PCR with one minute segments of 95 $^{\circ}$ C, 65 $^{\circ}$ C, and 72 $^{\circ}$ C, were followed by the addition of 3.2 units of Vent (exo-) polymerase and 27 additional cycles of 95 $^{\circ}$ C for one minute, 65 $^{\circ}$ C for two minutes, and 72 $^{\circ}$ C for three minutes. Uniamp primer sequence: 5'-CCTCTGAAGGTTCCAGAATCGATAG-3' (SEQ ID NO:6); Uniamp *XhoI* adapter sequence top strand: 5'-CCTCTGAAGGTTCCAGAATCGATAGCTCGAGT-3' (SEQ ID NO:7); bottom strand: 5'-P-ACTCGAGCTATCGATTCTGGAACCTTCAGAGGTTT-3' (SEQ ID NO:8).

Paragraph at page 17, line 27 - page 18, line 12

Reverse Transcription PCR. (See also Figure 2) One microgram of RNA was

reverse transcribed using the appropriate reverse PCR primer and superscript II at 50°C. For *iniA* and *asd*, three serial ten-fold dilutions of cDNA were made; 16S cDNA was diluted 1 in 10⁶, 1 in 10⁷, and 1 in 10⁸. PCR was performed with Taq polymerase and 1X PCR buffer (Gibco BRL) containing 2 mM MgCl₂ for 25 cycles annealing at 60°C for *iniA*; 35 cycles annealing at 58°C for *asd*; 25 cycles annealing at 63°C for 16S. PCR products were analyzed on a 1.7% agarose gel, images were stored to disk by digital camera (Appligene, Pleasanton, CA), and the amounts of PCR product were calculated by densitometry (Imaging Software, National Institute of Health, Bethesda, MD). Primers used for *iniA*: 5'-GCGCTGGCGGGAGATCGTCAATG-3' (SEQ ID NO:9), 5'-TGCGCAGTCGGGTCACAGGAGTCG-3' (SEQ ID NO:10); for *asd*: 5'-TCCCGCCGCCGAACACCTA-3' (SEQ ID NO:11), 5'-GGATCCGGCCGACCAGAGA-3' (SEQ ID NO:12); for 16S: 5'-GGAGTACGGCCGCAAGGCTAAAC-3' (SEQ ID NO:13), 5'-CAGACCCCGATCCGAACTGAGACC-3' (SEQ ID NO:14).

IN THE CLAIMS

46. (Amended) A method of determining whether a drug or compound is effective against *Mycobacterium tuberculosis* comprising:

- (a) transforming a vector construct comprising the nucleotide sequence of the *iniB* promoter inserted into a plasmid into a mycobacterium;
- (b) culturing the mycobacterium;
- (c) treating the cultured cells with the drug; and
- (d) measuring induction of the *ini*[A]*B* promoter, the presence of induction indicating the drug is effective against *Mycobacterium tuberculosis*.